(i) Twenty-five 3 to 4 week old laryngotracheitis susceptible chickens shall be injected intratracheally with 0.2 ml of vaccine rehydrated at the rate of 30 ml for 1,000 doses. Chickens shall be observed each day for 14 days. Deaths shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has five, six, or seven failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	4 or less	8 or more.
2	50	10 or less	11 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated or in lieu thereof, the serial declared unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method provided in paragraphs (c)(2) or (3) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.5}$ EID_{50} per dose for chicken embryo origin vaccine and $10^{2.0}$ EID_{50} or $10^{2.5}$ $TCID_{50}$ per dose for tissue culture origin vaccine.

[39 FR 44726, Dec. 27, 1974, as amended at 40 FR 18407, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 41 FR 44359, Oct. 8, 1976; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§ 113.329 Newcastle Disease Vaccine.

Newcastle Disease Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300, except § 113.34, and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in § 113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in § 113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Newcastle Disease susceptible chickens, all of the same age and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inject at least five embryos, 9 to 11 days old, in the allantoic cavity with at least 0.1 ml each. Disregard all deaths during the first 24 hours post-injection. To be a valid test, at least four embryos in

each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 7 days post-injection.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID_{50} by the Spearman-Karber or Reed-Muench method.

(3) Twenty to twenty-eight days postvaccination, all vaccinates and controls shall be challenged intramuscularly with at least $10^{4.0}$ EID_{50} of virus per chicken and observed each day for 14 days. Challenge virus shall be provided or approved by Animal and Plant Health Inspection Service.

(4) If at least 90 percent of the controls do not develop clinical signs of Newcastle disease during the observation period, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of Newcastle disease during the observation period, the Master Seed Virus is unsatisfactory.

(5) A strain identity test acceptable to Animal and Plant Health Inspection Service shall be conducted.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300, except §113.34, and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the vaccine judged accordingly.

(2) Safety test: Final container samples of completed product from each serial shall be tested to determine whether the vaccine is safe for use in susceptible young chickens. Vaccines recommended for use in chickens 10 days of age or younger shall be tested in accordance with paragraphs (d)(2)(i), (ii), and (iii) of this section.

(i) Twenty-five susceptible chickens, 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated by the eye drop method with the equivalent of 10 doses of vaccine and the chickens observed each day for 21 days. Severe respiratory signs or death shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has 3 failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and may be repeated.

(iv) Vaccines not recommended for use in chickens 10 days of age or younger shall be tested for safety as follows:

Each of twenty-five 3 to 5 week old Newcastle disease susceptible chickens shall be vaccinated as recommended on the label with the equivalent of ten doses and observed each day for 21 days. If any of the birds show severe clinical signs of disease or death during the observation period due to causes attributable to the product, the serial is unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a

virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{5.5}$ EID_{50} per dose.

[39 FR 44727, Dec. 27, 1974, as amended at 40 FR 18407, Apr. 28, 1975; 40 FR 23721, June 2, 1975; 40 FR 41090, Sept. 5, 1975; 42 FR 43618, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§ 113.330 Marek's Disease Vaccines.

Marek's disease vaccine shall be prepared from virus-bearing tissue culture cells. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300, and the requirements prescribed in this section. The identity test required in §113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Each lot of Master Seed Virus shall also be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(b) *Safety test.* The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(1) Specific pathogen free chickens or embryos, negative for Marek's disease virus antibodies, and from the same source, shall be isolated into the following groups:

(i) *Group 1.* At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) *Group 2.* At least 50 test subjects shall be injected with a very virulent Marek's disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek's disease in at least 80 per cent of the chickens within 50 days.

(iii) *Group 3.* Fifty uninoculated controls. For *in ovo* studies, this group should receive a sham inoculation of diluent.

(iv) *Group 4.* For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(2) At least 40 chickens in each group shall survive to 5 days of age. All chickens that die shall be necropsied and examined for lesions of Marek's disease and cause of death. The test shall be judged according to the following criteria:

(i) At 50 days of age, the remaining chickens in group 2 shall be killed and examined for gross lesions of Marek's disease. If at least 80 percent of this group do not develop Marek's disease, the test is inconclusive and may be repeated.

(ii) At 120 days of age, the remaining chickens in groups 1, 3, and 4 shall be weighed, killed, and necropsied. If less than 30 of the chickens in group 3 survive the 120 day period, or if any of the chickens in group 3 have gross lesions of Marek's disease at necropsy, the test is declared inconclusive. If less than 30 chickens in groups 1 and 4 survive the 120 day period; or if any of the chickens in groups 1 and 4 have gross lesions of Marek's disease at necropsy; or if the average body weight of the chickens in groups 1 or 4 is significantly (statistically) different from the average in group 3 at the end of the 120 days, the lot of Master Seed Virus is unsatisfactory.

(3) For tests involving *in ovo* inoculation, hatchability results shall also be reported for each group.

(c) *Immunogenicity.* Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity at the highest passage level allowed for the product, and the virus dose to be used shall be established as follows:

(1) Specific pathogen free chickens or embryos, negative for Marek's disease antibodies, and from the same source, shall be isolated into the following groups:

(i) *Group 1.* A minimum of 35 test subjects shall be inoculated with the vaccine, using the recommended route, at 1 day of age for chicks or 18 days of embryonation for embryos. The dose used shall be established by 5 replicate virus titrations conducted by a cell